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## FGF-8 Methods of Use

### **CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. provisional application Serial No. 60/416,377, filed October 4, 2002.

### **BACKGROUND**

5           The fibroblast growth factors (FGFs) are a group of structurally related peptides with at least 23 family members identified to date. FGFs specify the differentiation, patterning, and proliferation of a variety of tissues. FGF-8 is thought to play a role in limb bud patterning and development as well as midbrain development. In the early stages of embryogenesis, FGF-8 is expressed in developing brains, limbs, heart, lung, skeleton, teeth,  
10           and the renal system. Application of FGF-8 to embryos induces the formation of brains and ectopic limbs, consistent with the role of FGF-8 in developmental processes. FGF-8-null mice have an embryonic lethal phenotype. In adult tissues, low levels of expression have been detected in heart, brain, lung, kidney, testis, prostate, and ovary.

          The FGF-8 gene has 6 exons that potentially encode 8 isoforms. Seven isoforms of  
15           FGF-8 have been detected, with differences mapping to the N-terminal region (Blunt, A.G., et al. (1997) *J. Biol. Chem.* 272:3733-3738). The significance of these isoforms is unclear. FGF-8b displays the most potent mitogenic activity *in vitro* as compared to FGF-8a, -8c, -8d, -8e, -8f, and -8g isoforms (Blunt, A.G., et al., *supra*). FGF-8a does not stimulate mitogenesis through the known FGF receptors, unlike the other FGF-8 isoforms (Blunt, A.G., et al.,  
20           *supra*). FGF-8 has been shown to modulate chondrogenesis (Moftah M, et al. (2002) *Dev. Biol.* Sep 15; 249(2):270) but the involvement of FGF-8 in bone formation is unknown.

          FGF-8 is highly homologous to FGF-13, FGF-17, and FGF-18, containing more than 50% amino acid identity with each.

## SUMMARY

The present invention is based, in part, on the discovery that FGF-8 can stimulate proliferation of osteoblasts, which are known to play a role in mediating or modulating bone growth.

5 In one aspect, this invention features a method for treating a bone condition in a patient, e.g., a mammal, a human, a horse, a dog, or a cat. The method includes administering an effective amount of FGF-8, FGF-8 analog, or a FGF-8 agonist to the patient.

10 The patient can be at risk for, or suffering from a disease associated with excessive resorption or breakdown of bone tissue. Examples of such diseases include, but are not limited to, osteoporosis, osteopenia, bone defects, and osteogenesis imperfecta. The patient can also be suffering from bone loss as a result of immobility, bone fractures, malignancy, primary hyperparathyroidism, endocrine disorders, autoimmune arthritis, or addictive drug use. The patient can also be undergoing a treatment (e.g., corticosteroid treatment, bone  
15 marrow transplantation, or oophorectomy) known to result in bone loss. The term “bone condition” refers to any disease or symptom wherein osteoblast or osteoclast activity (or levels) is involved, and includes any of the diseases or situations described above.

As used herein, “FGF-8a” is an isolated polypeptide of 204 amino acids in length. It includes mouse FGF-8a, rat FGF-8, and human FGF-8a, the sequences of which are shown  
20 below.

Mouse FGF-8a:

MGSPRSALSCLLLHLLVLCLQAQHVREQSLVTDQLSRRLIRTYQLYSRTSGKHVQVL  
ANKRINAMAEDGDPFAKLIVETDTFGSRVRVRGAETGLYICMNKKGKLIAKSNGKG  
25 KDCVFTEIVLENNYTALQNAKYEGWYMAFTRKGRPRKGSKTRQHQREVHFMKRLP  
RGHHTTEQSLRFEFLNYPPFTRSLRGSQRTWAPEPR (SEQ ID NO: 1)

Rat FGF-8:

MGSPRSALSCLLLHLLVLCLQAQHVREQSLVTDQLSRRLIRTYQLYSRTSGKHVQVL  
30 ANKRINAMAEDGDPFAKLIVETDTFGSRVRVRGAETGLYICMNKKGKLIAKSNGKGK

DCVFTEIVLENNYTALQNAKYEGWYMAFTRKGRPRKGSKTRQHQRVHFMKRLPR  
GHHTTEQSLRFEFLNYPPFTRSLRGSQRTWAPEPRL (SEQ ID NO:2)

Human FGF-8:

5 MGSPRSALSCLLLHLLVLCLQAQHVREQSLVTDQLSRRLIRTYQLYSRTSGKHVQVL  
ANKRINAMAEDGDPFAKLIVETDTFGSRVRVRGAETGLYICMNKKGKLIASNGKKG  
DCVFTEIVLENNYTALQNAKYEGWYMAFTRKGRPRKGSKTRQHQRVHFMKRLPR  
GHHTTEQSLRFEFLNYPPFTRSLRGSQRTWAPEPR (SEQ ID NO: 3).

10 The mouse FGF-8a DNA sequence is as follows:

CGCACCTTCGGCTTGTCCCCCGCGGCCTCCAGTGGGACGGCGTGACCCC  
GCTCGGGCTCTCAGTGCTCCCGGGGCGCGGCCATGGGCAGCCCCCGCT  
CCGCGCTGAGCTGCCTGCTGTTGCACTTGCTGGTTCTCTGCCTCCAAGCC  
CAGCATGTGAGGGAGCAGAGCCTGGTGACGGATCAGCTCA GCCGCCGCCT  
15 CATCCGGACCTACCAGCTCTACAGCCGCACCAGCGGGAAGCACGTGCAGG  
TCCTGGCCAACAAGCGCATCAACGCCATGGCAGAAGACGGAGACCCCTTC  
GCGAAGCTCATTGTGGAGACCGATACTTTTGAAGCAGAGTCCGAGTTCG  
CGGCGCAGAGACAGGTCTCTACATCTGCATGAACAAGAAGGGGAAGCTAA  
TTGCCAAGAGCAACGGCAAAGGCAAGGACTGCGTATTCACAGAGATCGTG  
20 CTGGAGAACAACACTACACGGCGCTGCAGAACGCCAAGTACGAGGGCTGGTA  
CATGGCCTTTACCCGCAAGGGCCGGCCCCGCAAGGGCTCCAAGACGCGCC  
AGCATCAGCGCGAGGTGCACTTCATGAAGCGCCTGCCGCGGGGCCACCAC  
ACCACCGAGCAGAGCCTGCGCTTCGAGTTCCTCAACTACCCGCCCTTCAC  
GCGCAGCCTGCGCGGCAGCCAGAGGACTTGGGCCCCGGAGCCCCGATAGG  
25 CGCTCGCCCAGCTCCTCCCCACCCAGCCGGCCGAGGAATCCAGCGGGAGC T  
CG

(SEQ ID NO:4; see also Genbank® GI No. 619919)

The rat FGF-8 DNA sequence is as follows:

30 ATGGGCAGCCCCCGCTCCGCGCTGAGCTGCCTGCTGTTGCACTTGCTGGT  
TCTCTGCCTCCAAGCCCAGCATGTGAGGGAGCAGAGCCTGGTGACGGATC

AGCTCAGCCGCCGCCTCATCCGGACCTACCAGCTCTACAGCCGCACCAGC  
 GGGAAGCACGTGCAGGTCCTGGCCAACAAGCGCATCAACGCCATGGCAGA  
 AGACGGAGACCCCTTCGCAAAGCTCATTGTGGAGACCGATACTTTTGAA  
 GCAGAGTCCGAGTCCGCGGAGCAGAGACCGGTCTGTACATCTGCATGAACAAGA  
 5 AGGGGAAGCTAATCGCCAAGAGCAACGGCAAAGGCAAGGACTGCGTGTTACG  
 GAGATCGTGCTGGAGAACAACACTACACGGCGCTGCAGAACGCCAAGTACGA  
 GGGCTGGTACATGGCCTTTACCC GCAAGGGCCGGCCCCGCAAG GGTCCAAGA  
 CGCGCCAGCACCAGCGCGAGGTGCACTTCATGAAGCGCCTGCCGCGGGGC  
 CACCACACCACAGAGCAGAGCCTCCGCTTCGAGTTCCTCAACTACCCGCC  
 10 CTTACGCGCAGCCTGCGCGGCAGCCAGAG GACTTGGGCCCCGGAGCCCC  
 GATAG  
 (SEQ ID NO:5; see also Genbank® GI No. 18461160)

The sequence of human FGF-8a is as follows:

15 ATGGGCAGCCCCCGCTCCGCGCTGAGCTGCCTGCTGTTGCACTTGCTGGT  
 CCTCTGCCTCCAAGCCCAGCATGTGAGGGAGCAGAGCCTGGTGACGGATC  
 AGCTCAGCCGCCGCCTCATCCGGACCTACCAACTCTACAGCCGCACCAGC  
 GGGAAGCACGTGCAGGTCCTGGCCAACAAGCGCATCAACGCCATGGCAGA  
 GGACGGCGACCCCTTCGCAAAGCTCATCGTGGAGACGGACACCTTTGGAA  
 20 GCAGAGTCCGAGTCCGAGGAGCCGAGACGGGCCTCTACATCTGCATGAAC  
 AAGAAGGGGAAGCTGATCGCCAAGAGCAACGGCAAAGGCAAGGACTGCGT  
 CTTACGGAGATTGTGCTGGAGAACAACACTACACAGCGCTGCAGAATGCCA  
 AGTACGAGGGCTGGTACATGGCCTTCACCCGCAAGGGCCGGCCCCGCAAG  
 GGCTCCAAGACGCGGCAGCACCAGCGTGAGGTCCACTTCATGAAGCGGCT  
 25 GCCCCGGGGCCACCACACCACCGAGCAGAGCCTGCGCTTCGAGTTCCTCA  
 ACTACCCGCCCTTCACGCGCAGCCTGCGCGGCAGCCAGAGGACTTGGGCC  
 CCGGAGCCCCGATAG

(SEQ ID NO: 6; see also Genbank® GI No. 1184864)

30 Analogs of FGF-8 include functional equivalents of FGF-8 (e.g., functional  
 equivalents of mouse FGF-8, human FGF-8, or rat FGF-8). In terms of FGF-8 itself,  
 functional equivalents include all proteins which are immunologically cross-reactive with

and have substantially the same function as FGF-8 (e.g., any of SEQ ID NOs: 1-3). That equivalent may, for example, be a fragment of FGF-8 containing a subsequence of amino acids (e.g., a truncation) and including a FGF-8 active site or sites, a substitution, addition or deletion mutant of FGF-8, or a fusion of FGF-8 or a fragment or a mutant with other amino acids.

A “FGF-8 agonist” is a compound which (1) has a high affinity (e.g., a  $K_i$  of  $10^{-7}$  -  $10^{-9}$  M, a  $K_i$  of  $10^{-8}$  -  $10^{-9}$  M) for a FGF-8-binding receptor (as defined by the receptor binding assay described in Motulsky, H.J and Mahan, L.C. (1984). *Mol. Pharmacol.* 25: 1; and (2) promotes the proliferation of bone cells, e.g., osteoblasts.

In one embodiment, the methods described herein include administering to a patient an effective amount of FGF-8 having the amino acid sequence of SEQ ID NO: 1, 2, or 3.

In another embodiment, the method includes administering to a patient an effective amount of a FGF-8 agonist having a fragment (e.g., any sequence between about 10 and 200, alternatively between about 10 and 100, alternatively between about 10 and 50, alternatively between about 10 and 25 amino acids in length, inclusive, of SEQ ID NO: 1, 2, or 3); or the entirety of the amino acid sequence of SEQ ID NO: 1, 2, or 3. For example, a FGF-8 agonist is a peptide being less than 87 amino acids in length, e.g., a peptide having less than 30 amino acids, or more than 10 (e.g., any integer between 10 and 90, inclusive) amino acids, and containing, in consecutive sequence, any part of SEQ ID NO: 1, 2, or 3.

In a further embodiment, the method includes administering to a patient an effective amount of a FGF-8 agonist containing an amino acid sequence that is at least 60% (e.g., 70%, 80%, 90%, 95%, or 98%) identical to SEQ ID NO: 1, 2, or 3. The “percent identity” of two amino acid sequences can be determined using the algorithm of Karlin and Altschul (1990, *Proc. Natl. Acad. Sci. USA* 87: 2264-2268), modified as in Karlin and Altschul (1993, *Proc. Natl. Acad. Sci. USA* 90: 5873-5877). Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the peptide molecules described herein. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25(17): 3389-3402. When utilizing

BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

In still another embodiment, the method includes administering to a patient an effective amount of a FGF-8 agonist containing SEQ ID NO: 1, 2, or 3 with up to 14 (e.g.,  
5 any integer between 1 and 14, inclusive) conservative amino acid substitutions. A “conservative amino acid substitution” is one in which an amino acid residue is replaced with another residue having a chemically similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid,  
10 glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Amino acid analogs (e.g., phosphorylated amino acids) are also contemplated in  
15 the present invention.

In another aspect, this invention features a method for increasing or maintaining bone density. The method includes administering to a subject (e.g., a mammal, a human, a horse, a dog, or a cat) in need thereof an effective amount of FGF-8, FGF-8 analog, or a FGF-8 agonist as described herein. As used herein, the subject may have a substantially normal  
20 bone density or the subject may be at risk of bone deterioration. Examples of these subjects include postmenopausal women, usually at age 50 and over, and men over 60 years of age.

In another aspect, the invention features a method for treating or preventing an FGF-8-mediated bone disease. The method includes administering to a subject (e.g., a mammal, a human, a horse, a dog, or a cat) in need thereof an effective amount of FGF-8, FGF-8 analog,  
25 or a FGF-8 agonist as described herein.

In a further aspect, this invention features a method for stimulating osteoblast growth or modulating osteoblast apoptosis. The method includes administering to a subject in need thereof an effective amount of FGF-8, FGF-8 analog, or a FGF-8 agonist. The term  
“osteoblast” refers to bone-forming cells.

30 This invention also features an article of manufacture that includes a vessel containing FGF-8, a FGF-8 analog, a FGF-8 agonist, or a nucleic acid encoding FGF-8, a

FGF-8 analog, or a FGF-8 agonist; and instructions for use of FGF-8, FGF-8 analog, or a FGF-8 agonist for treatment of a bone condition by administering an effective amount of FGF-8, FGF-8 analog, or a FGF-8 agonist to a patient.

Also within the scope of this invention is an article of manufacture. The article includes packaging material; and contained within the packaging material, FGF-8, FGF-8 analog, or a FGF-8 agonist. The packaging material comprises a label that indicates that FGF-8, FGF-8 analog, or a FGF-8 agonist can be used for treating a bone condition (e.g., osteoporosis, osteopenia, bone defects, or osteogenesis imperfecta) in a patient. In other aspects, the label includes dosage information.

Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

### DESCRIPTION OF DRAWINGS

FIG. 1 depicts the effect of various concentrations of FGF-8a or vehicle on thymidine incorporation by rat osteoblasts. \*\* represents  $p < 0.01$ , and \* represents  $p < 0.05$ .

FIG. 2 depicts the effect of various concentrations of FGF-8a or vehicle on cell thymidine incorporation by osteoblastic UMR-106 cells. \*\* represents  $p < 0.01$ .

FIG. 3 depicts the effect of various concentrations of FGF-8a or vehicle on osteoclast formation from mouse bone marrow cultures. \* represents  $p < 0.05$ , and \*\* represents  $p < 0.01$ .

### DETAILED DESCRIPTION

This invention relates to use of FGF-8, a FGF-8 analog, or a FGF-8 agonist for stimulating osteoblast growth or modulating osteoblast apoptosis. FGF-8, as well as a FGF-8 analog or FGF-8 agonist, also can be prepared by a synthetic method. More specifically, synthesis of peptides (e.g., peptides derived from FGF-8) is well established in the art. See, e.g., Stewart, *et al.* (1984) *Solid Phase Peptide Synthesis* (2<sup>nd</sup> Ed.); and Chan (2000) "Fmoc Solid Phase Peptide Synthesis, A Practical Approach," Oxford University Press. The peptides may be synthesized using an automated peptide synthesizer (e.g., a Pioneer™ Peptide Synthesizer, Applied Biosystems, Foster City, CA). For example, a peptide is prepared on methylbenzylhydramine resin followed by hydrogen fluoride deprotection and cleavage from the resin. The synthesized peptide can be further purified by a method such as

affinity column chromatography or high pressure liquid chromatography. Standard physicochemical characterization techniques are known in the art, including NMR ( $^{13}\text{C}$ ,  $^1\text{H}$ ,  $^{19}\text{F}$ , or  $^{31}\text{P}$ ) and IR, which can provide confirmatory evidence of the identity and purity of the synthetic products. Amino acid analysis can also be used to confirm the amino acid composition of the peptide. Laser desorption mass spectroscopy can be used to identify the molecular weight of synthetic products.

One aspect of this invention is a method for treating a bone condition with an effective amount of a FGF-8, FGF-8 analog, or a FGF-8 agonist. Another aspect of this invention is a method for increasing or maintaining bone density with a FGF-8, FGF-8 analog, or a FGF-8 agonist. The term “treating” is defined as the application or administration of a composition including a FGF-8, FGF-8 analog, or a FGF-8 agonist to a patient, who has, or is determined to have, a bone condition, a symptom of a bone condition, a disease or disorder secondary to a bone condition, or a predisposition toward a bone condition, with the purpose to cure, alleviate, relieve, remedy, or ameliorate the bone condition, the symptom of the bone condition, the disease or disorder secondary to the bone condition, or the predisposition toward the bone condition.

“An effective amount” refers to an amount of FGF-8, FGF-8 analog, or a FGF-8 agonist that confers a therapeutic effect on the treated subject. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). An effective amount of FGF-8, FGF-8 analog, or a FGF-8 agonist described above may range from about 1  $\mu\text{g/Kg}$  body weight to about 1000  $\mu\text{g/Kg}$  body weight. Effective doses will also vary depending on the route of administration, as well as the possibility of co-usage with other agents for stimulating osteoblast growth or modulating osteoblast apoptosis, such as a bone anti-resorptive agent (e.g., calcitonin or bisphosphonate) or a bone anabolic agent (e.g., parathyroid hormone, parathyroid hormone related protein, cytokines, or growth hormone).

As used herein, FGF-8, FGF-8 analog, and FGF-8 agonists are defined to include pharmaceutically acceptable derivatives (e.g., salts).

The methods delineated herein can also include the step of identifying that the subject is in need of treatment for the aforementioned disorders or condition. The identification can be in the judgment of a subject or a health care professional and can be a subjective (e.g.,

opinion) or objective (e.g., measurable by a test or diagnostic method).

The methods of treating delineated herein can include use or administration of a nucleic acid that encodes for FGF-8 (including all isoforms thereof), FGF-8 analog, or FGF-8 agonist, including those nucleic acids delineated as SEQ ID NOs. 4-6. See also, C.A.

5 MacArthur et al., *J. of Virology*, (1995) 2501-2507. The nucleic acids described herein can be incorporated into gene constructs to be used as a part of a gene therapy protocol to deliver nucleic acids encoding FGF-8 (including all isoforms thereof), FGF-8 analog, or FGF-8 agonist. The invention features expression vectors for in vivo transfection and expression of a polypeptide described herein in particular cell types so as to reconstitute the function of, or  
10 alternatively, antagonize the function of a cell, relating to osteoblast or osteoclast function, and bone conditions. Expression constructs of such components may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the component gene to cells, preferably adipose cells, in vivo. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus,  
15 adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (e.g. antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or CaPO<sub>4</sub> precipitation carried out in  
20 vivo. Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes in vivo, particularly into humans.

In clinical settings, the gene delivery systems for the therapeutic gene can be introduced into a patient by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced  
25 systemically, e.g. by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene  
30 delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery system

can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate, trifluoroacetate, and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)<sub>4</sub><sup>+</sup> salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

Also within the scope of this invention is a pharmaceutical composition that contains an effective amount of FGF-8, FGF-8 analog, or a FGF-8 agonist, and a pharmaceutically acceptable carrier. These compositions are suitable for use in the methods delineated herein.

The term "pharmaceutically acceptable carrier" refers to a carrier (adjuvant or vehicle) that may be administered to a patient, together with FGF-8, FGF-8 analog, or a FGF-8 agonist, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver FGF-8, FGF-8 analog, or a FGF-8 agonist.

Pharmaceutically acceptable carriers that may be used in the pharmaceutical compositions described above include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d- $\alpha$ -tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as

human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl- $\beta$ -cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein. Oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents, which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions.

FGF-8, a FGF-8 analog, or an FGF-8 agonist can be modified to increase stability or in vivo half life by linkage with a lipid, a carbohydrate, or other polymer. For example, FGF-8, a FGF-8 analog, or an FGF-8 agonist can be conjugated to a water soluble polymer, e.g., hydrophilic polyvinyl polymers. Such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG).

To practice the method for treating a bone condition or the method for increasing or maintaining bone density, FGF-8, FGF-8 analog, or a FGF-8 agonist can be administered to a patient or a subject. The FGF-8, FGF-8 analog, or the FGF-8 agonist can, for example, be administered in a pharmaceutically acceptable carrier such as physiological saline, in combination with other drugs, and/or together with appropriate excipients. It also can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously; or orally, buccally, nasally, transmucosally, topically, in an ophthalmic preparation, by inhalation, by intracranial injection or infusion techniques. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Lower or higher doses than those described above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors,

including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

5           A pharmaceutical composition may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents  
10       include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

          A sterile injectable composition (e.g., aqueous or oleaginous suspension) can be  
15       formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents.

          Topical administration of a pharmaceutical composition is useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable  
20       ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active  
25       compound suspended or dissolved in a carrier with suitable emulsifying agents. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation.  
30       Topically-applied transdermal patches are also included in this invention.

A pharmaceutical composition may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

FGF-8 agonists can be tested for their abilities to stimulate osteoblast growth, modulate osteoblast apoptosis, or modulate osteoclast formation by examining their activities in the *in vitro* assays described herein. See the specific examples below. *In vivo* screening can also be performed by following procedures well known in the art. See, e.g., Cornish *et al.* (1997) *Am J Physiol* 273: E1113-E1120; and Cornish *et al.* (2000) *Am J Physiol* 279: E730-E735.

An effective amount of a compound described herein, or a composition described herein, can be administered to a subject (including a subject identified as in need of such treatment) to produce such effects as those described herein.

All references cited herein, whether in print, electronic, computer readable storage media or other form, are expressly incorporated by reference in their entirety, including but not limited to, abstracts, articles, journals, publications, texts, treatises, internet web sites, databases, patents, and patent publications.

The invention will be further described in the following examples. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

Example 1. Promoting proliferation of bone cells

*Osteoblast-Like Cell Culture.* Osteoblasts were isolated from 20 day fetal rat calvariae as previously described (Cornish *et al.* (1999) *American Journal of Physiology - Endocrinology & Metabolism* 277: E779-E783). Briefly, calvariae were excised and the frontal and parietal bones, free of suture and periosteal tissue, were collected. The calvariae were sequentially digested using collagenase and the cells from digests 3 and 4 were collected, pooled and washed. Cells were grown to confluence and then subcultured into 24 well plates. Cells were growth arrested in minimum essential medium (MEM)/0.1% bovine serum albumin for 24 h. Fresh media and experimental compounds were added for a further 24 h. Cells were pulsed with tritiated-thymidine two hours before the end of the

experimental incubation. The effect of FGF-8a on osteoblast proliferation was assessed by the measurement of [<sup>3</sup>H]-thymidine incorporation into isolated primary osteoblasts and osteoblast-like cells.

As shown in FIGs. 1 and 2, FGF-8, in a dose-dependent manner, stimulated the proliferation ([<sup>3</sup>H]-thymidine incorporation) of primary fetal rat osteoblasts and osteoblast-like cell lines at concentrations of > 5 ng/ml.

Example 2. Inhibiting formation of osteoclasts

*Osteoclastogenesis assay.* Bone marrow is obtained from the long bones of normal mice, aged 4-6 weeks were cultured. Non-adherent cells are removed and the cultures are grown in the presence of 1 $\alpha$ ,25-dihydroxyvitamin D3 throughout the experiment. The cultures were maintained for 7 days and the number of tartrate-resistant acid phosphatase-positive multinucleated cells was assessed. As shown in FIG. 3, FGF-8a inhibited the formation of osteoclasts in bone marrow cultures in a dose-dependent manner.

Example 3. Expression of FGF-8 in fetal rat brains and cultured osteoblasts

*RT-PCR.* RNA was collected. Briefly, RNA was extracted from primary fetal rat osteoblasts using a RNA extraction kit (Qiagen). RNA was quantitated by spectrophotometry and stored at -20°C until further use. Semi-quantitative RT-PCR was performed using standard techniques to demonstrate the expression of the FGF-8 gene in the osteoblasts. Analysis of RT-PCR products by agarose gel electrophoresis showed that FGF-8 is expressed in fetal rat brain and cultured rat osteoblasts.

## OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.